

The role of prebiotic and probiotics in poultry gut health

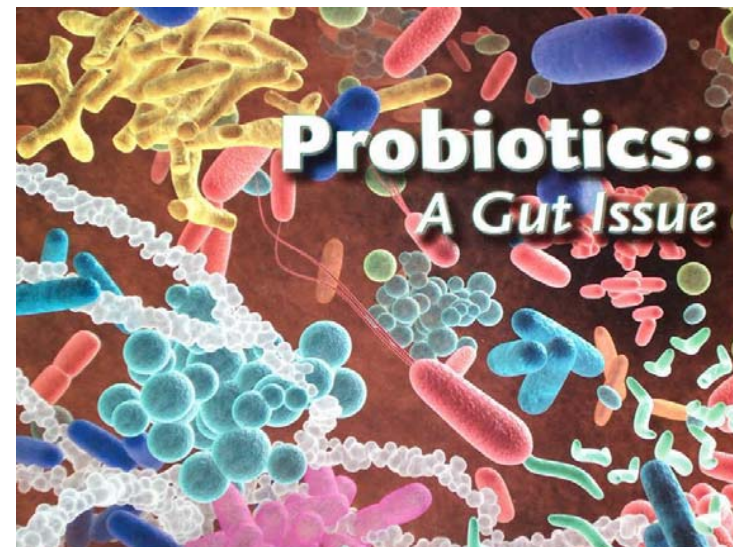
WPSA –Italy

Forli , 7 April 2011

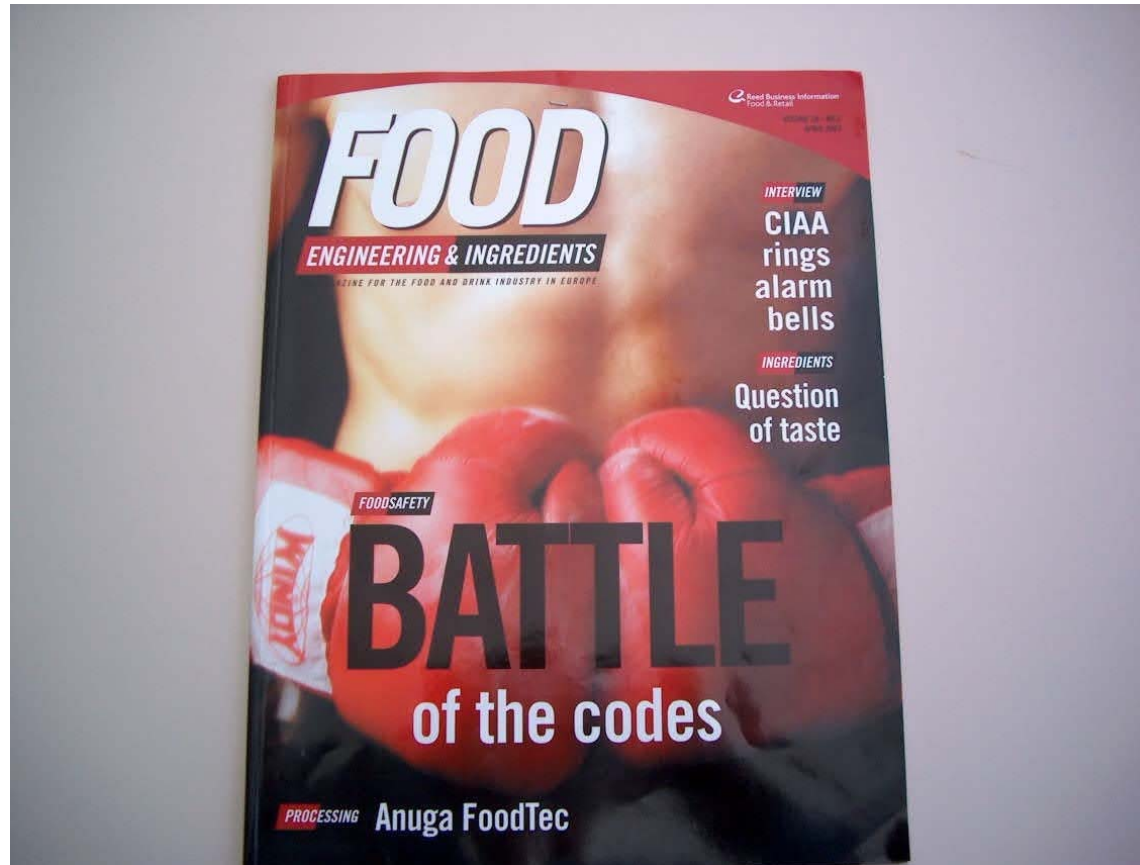
Joaquim Brufau & Borja Vila,

IRTA

Centre Mas de Bover, Spain



Have a nice day



Experiences
Dossiers
Authorization of
Probiotics and
Prebiotics

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Regulation 1831/2003



Dossiers of all feed additives have to be presented to the European Commission for re-registration before 7 November 2010. Dossiers not filed will get no approval and the feed additive has to be taken off the market.

Definition of Feed Additive

Substances, micro-organisms or preparations, other than feed material and premixtures, which are intentionally added to **feed** or **water** in order to perform, in particular, one or more of the functions mentioned in Article 5(3)

Conditions for Authorisation

Efficacy

- ✓ Favourably affect the characteristics of feed or animal products
- ✓ Favourably affect the colour of ornamental fish and birds
- ✓ Satisfy the nutritional needs of animals
- ✓ Favourably affect animal production, performance or **welfare**
- ✓ Have a coccidiostat or histomonostatic effect

Categories of Feed Additives

- **Technological additives:** any substance added to feed for a technological purpose
- **Sensory additives:** any substance, the addition of which to feed improves or changes the organoleptic properties of the feed, or the visual characteristics of the food derived from animals
- **Nutritional additives**
- **Zootechnical additives:** additives used to affect favourably the performance of animals in **good health** or the environment
- **Coccidiostats and histomonostats**

The category '**zootechnical additives**' is divided into **4 functional groups**:

(a) **digestibility enhancers**: substances which, when fed to animals, increase the digestibility of the diet, through action on target feed materials;

(b) **gut flora stabilisers**: micro-organisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora;

(c) substances which favourably affect **the environment**;

(d) **other** zootechnical additives.

the Specific Mechanical Energy (SME) control remains available through the hydraulically driven cone system. Advantage of the Crown outlet is that coarse particles remain coarse contrary to products being pelleted with a pelleting press.

Great Feed Additives

Welcome to our Company.
Here's your new work place. Make sure you
get These dossiers To The Commission
by early November!



China near Shanghai for the production of its sweetener Sucram.

Lonza

Swiss ingredients manufacturer Lonza will locate its new vitamin B3 manufacturing site at Nansha to become the leading high-tech life science plant in China.

Selko

Selko Feed Additives, a Nutreco company in the Netherlands, is investing €6 million to upgrade and increase capacity.

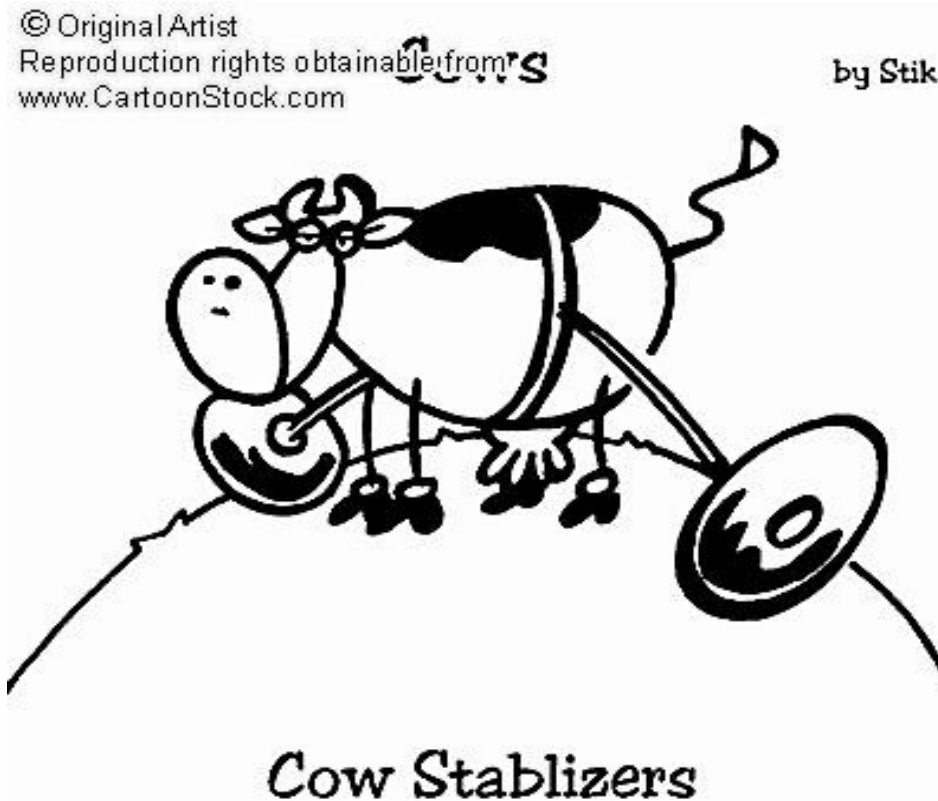
Kemin

Kemin Industries is establishing two production facilities in Italy for the production of spray-dried and liquid pet food palatants.

<http://www.>

For up-to-date news on the feed industry visit www.AllAboutFeed.net/news

A zootechnical additive is any additive used to favourably affect the performance of animals in good health, or to favourably affect the environment



New European model of Animal feeding

AP should be sustainable in the EU and based on:

- 1) Animal Protection & **Animal welfare.**
- 2) Consumer Protection.
- 3) Environment protection.

How to overcome this problem at farm level

- 1.- Improve management of animals.
- 2.- Feeding programs and feed composition.
- 3.- Supplementation of diets with alternative additives to AGP.

Additives AGP forbidden since 2006

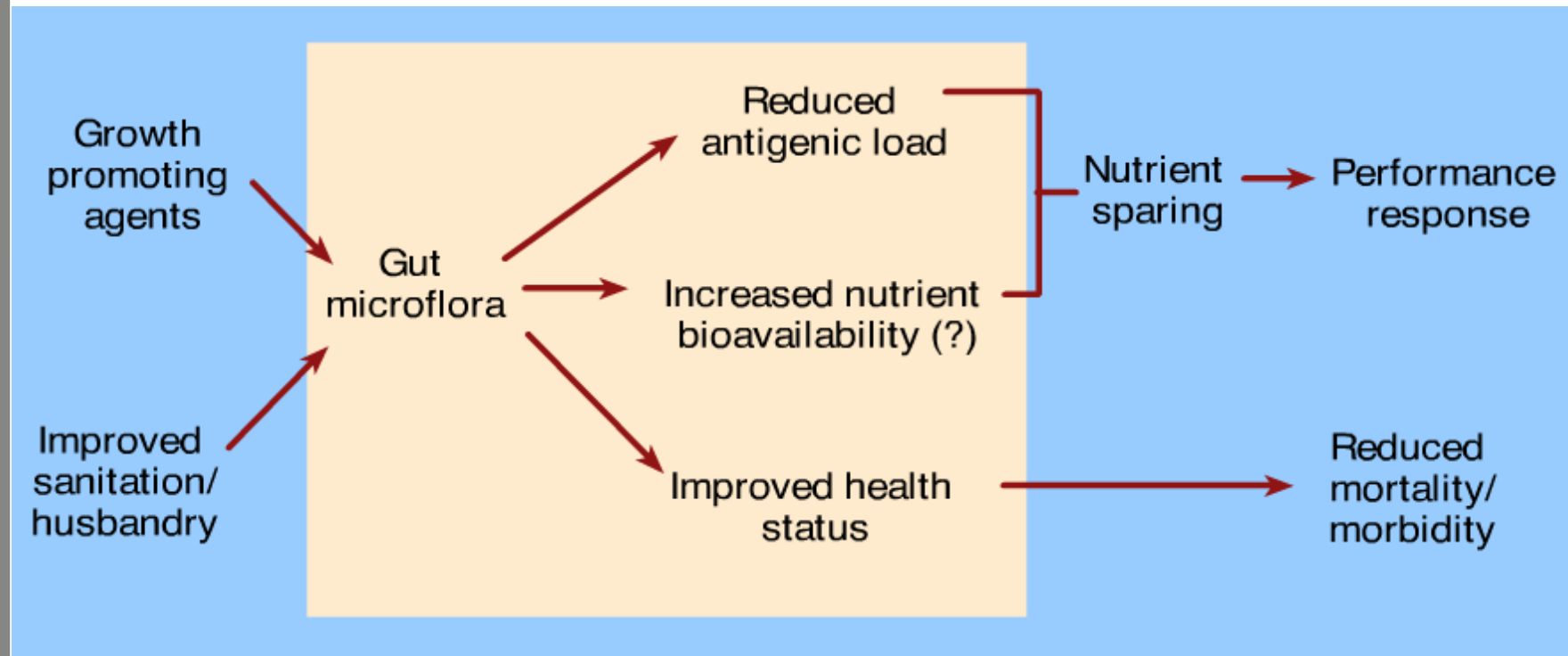


Alternatives feed additive products:

- Organic acids.
- Enzymes preparations.
- **Micro-organisms (Probiotics).**
- **Oligosaccharides (Prebiotics).**
- **Immunity enhancers.**
- Highly available minerals.
- Herbs and essential oils.



Animal nutrition and Gut microflora interactions (**Animal protection**).



Andrew Chesson

Feed additives with capacity for balancing intestinal microflora

Category Gut flora stabilizers

- **Prebiotics (Oligosaccharides)**
- **Microorganism (Probiotics)**

Definitions of :

Probiotic is live microbial feed supplemented which beneficially affect the host animal by improving its intestinal balance (Fuller 1989)

Prebiotic is any food component that escapes digestion in the small intestine enters the lower gut, where it may serve a growth substrate for intestinal bacteria (Gibson and Roberfroid, 1995).

Probiotics: *A Gut Issue*

Microorganism (Probiotics)

**European Union Register of Feed Additives
pursuant to Regulation (EC) No 1831/2003**

**Appendixes 3b & 4.
Annex : List of additives**

(Status: Released 9 March 2011.)

Edition  113

Directorate D - Animal Health and Welfare

Unit D2 – Feed

32 Authorisations in EU as Gut flora stabilizer and 4 as others zootechnical

Bacillus cereus var. Toyoi
Enterococcus faecium
Pediococcus acidilactici
Bacillus amyloliquefaciens
Bacillus subtilis

Saccharomyces cerevisiae
Lactobacillus rhamnosus
Lactobacillus fracciminis

Prebiotic

Oligosaccharides → Hexose monosaccharides with a polymerisation degree between 2 and 20

FOS = fructo-oligosaccharide

Encourage growth of *lactobacillus*, *bifidobacterium*,
suppress growth of *salmonella*

MOS = mannan-oligosaccharide

Poorly fermentable sugar. Absorb enteric pathogens.
Immunomodulation

GOS = galacto-oligosaccharide

XOS = xylo-oligosaccharide

In relation to the nutritional, metabolic and immunological point of view, according to Vanbelle *et al.*, (1990) an ideal probiotic microorganism desirably must fulfil the following requirements:

- Be resistant against digestive enzymes, lysozyme, the low pH in the stomach for a few hours, also to bile salts;
- Produce a sufficient decrease in the pH of the gut to avoid the development of pathogens and reduce the production of toxins;
- Produce antibiotics and be resistant to in feed antimicrobials (coccidiostats);
- Attach to the brush border cells or colonization of mucous and glycocalix, although this characteristic is not strictly necessary;
- Be present in a viable state resistant to product/feed processing and storage; and confer immune stimulation to the host.

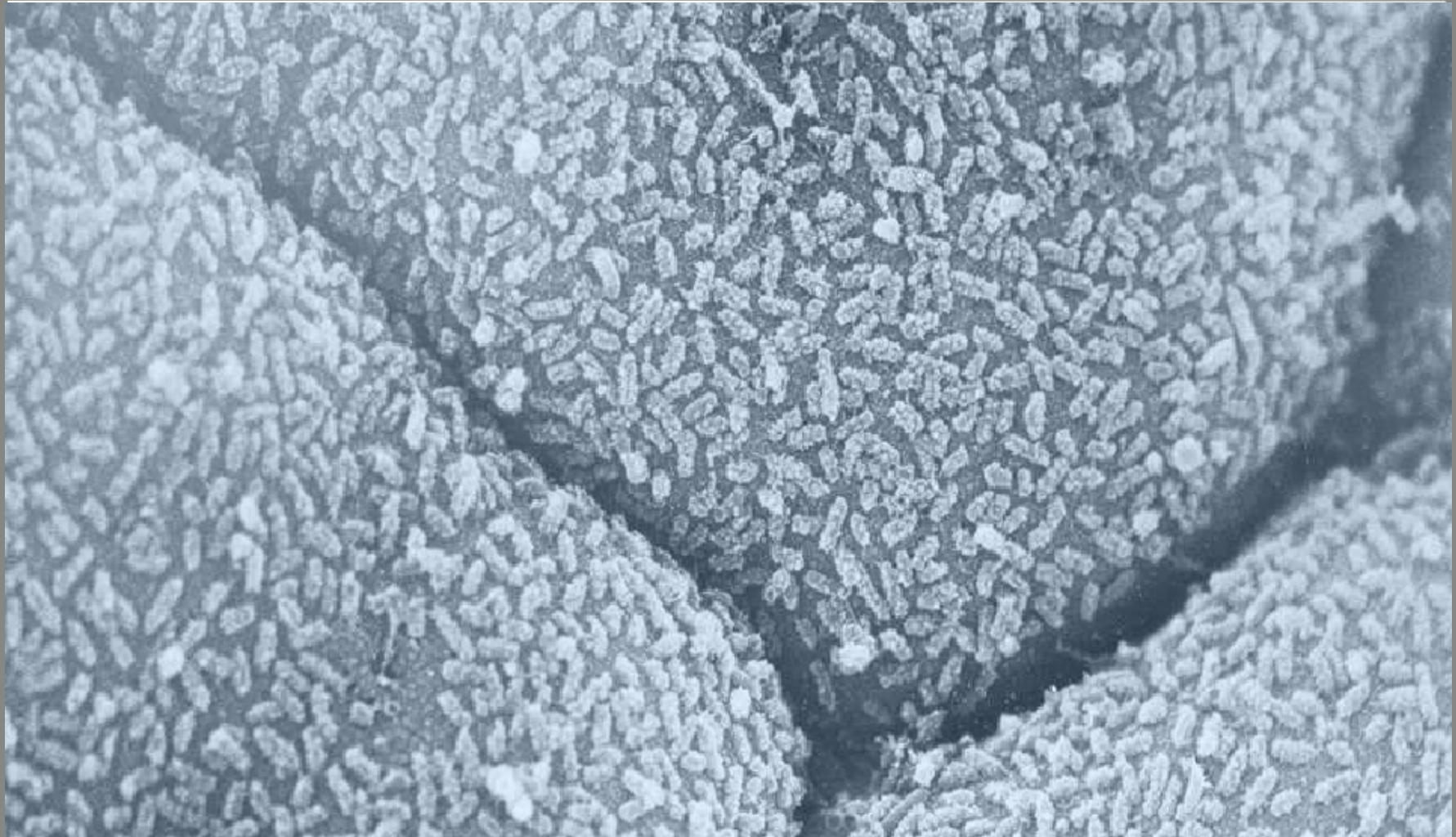
The main ideal characteristics of prebiotic, according Simmering and Blaut 2001 are:

- **Be neither hydrolyzed nor absorbed by mammalian enzymes or tissues.**
- **Selectively enrich for one or limited number of beneficial bacteria.**
- **Beneficially alter the intestinal microbiota and their activities.**
- **Beneficially alter luminal or systemic aspects of the host defense system**

How to determine the efficacy of new substances

Technological challenge

“Molecular biology and implications
on the efficacy assessment of
alternatives products to AGP”



How much do we know about
intestinal microbiota?



Year	1953	1963	1973	1983	1993	2002
Body weight, kg	1.45	1.59	1.77	1.93	2.05	2.42
Days	73	67	60	49	42	42



24 d age	2001- Strain	1957-Strain
Body weight, g	693 ^a	201 ^b
Thymus, %*	0.24	0.30
Bursa of Fabricius, %	0.29 ^b	0.46 ^a
Spleen, %	0.12 ^b	0.18 ^a
Cecal tonsils, %	0.03 ^b	0.04 ^a



European Food Safety Authority

The EFSA Journal (2008) 920, 1-19

SCIENTIFIC OPINION

Functional groups of additives as described in Annex 1 of Regulation (EC) No 1831/2003¹

Self-task of the Panel on Additives and Products or Substances used in Animal Feed

(EFSA-Q-2007-173)

Adopted on 11 December 2008

Examples of Self-tasks in FEEDAP

The assessment of herbs , essential oils and other plant products as “ additive” for use in animal nutrition.

Functional groups for Zootechnical additives

Compatibility of microbial additive with a substance showing antimicrobial activity.

Functional groups for Zootechnical additives (1)

Potential New categories :

Welfare additives: *any additive used to favourably affect the welfare of animals.*

with the following functional groups

Metabolic regulators: *substances which act within the animal to correct undesired consequences of nutritional origin.*

Immuno-modulators: *agents or substances which positively influence the immune function of the animal.*

Detoxifiers: *agents or substances which degrade or otherwise reduce the toxicity of contaminants ingested with feedstuffs*

Other welfare additives

Functional groups for Zootechnical additives (2)

Potential new categories:

2. Additive which improve product quality

with the following functional groups

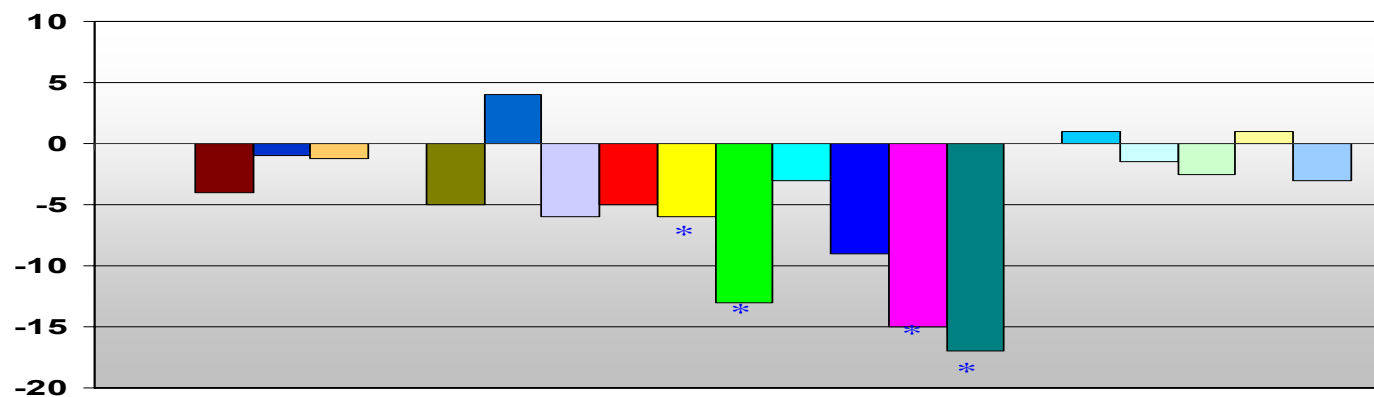
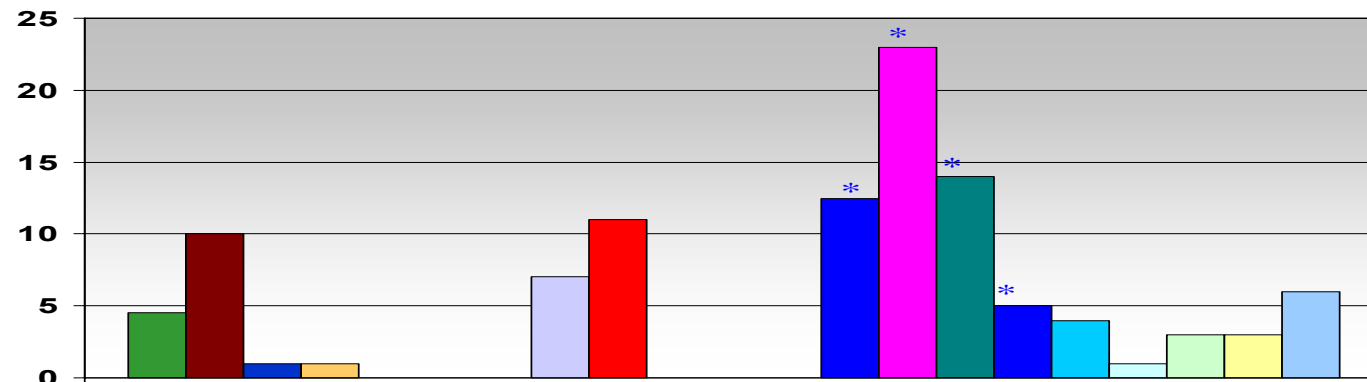
Pathogen reducers – additives intended to reduce the numbers of zoonotic pathogens in animal food products.

Nutrition enhancers – additives intended to improve nutritional characteristics of the animal products.

Sensory additives – additives intended to improve the sensory characteristics and product acceptance of animal products.

Other product quality additives

Relative effects of probiotics on performance parameters (% of control) in broilers and turkeys



AGPs: what benefits were they delivering in numbers?

	AGPs	Enzymes	Microbials	Mannoprotein
N° trials	5159	2557	234	34
Duration (days)	41	30	36	42
LW control	1075	1043	1331	2149
LW dif.	40 (129)	54 (147)	25 (192)	39 (108)
FCR control	2.16	1.99	1.87	1.88
FCR dif.	-0.073 (164)	-0.105 (185)	-0.030 (195)	-0.042 (112)
Improvement frequency (%)	74	75	70	79

AGPs + enzymes + microorganisms : 4.1% FCR / 4.0% in WG₈₀

Some examples of efficacy .

Probiotics

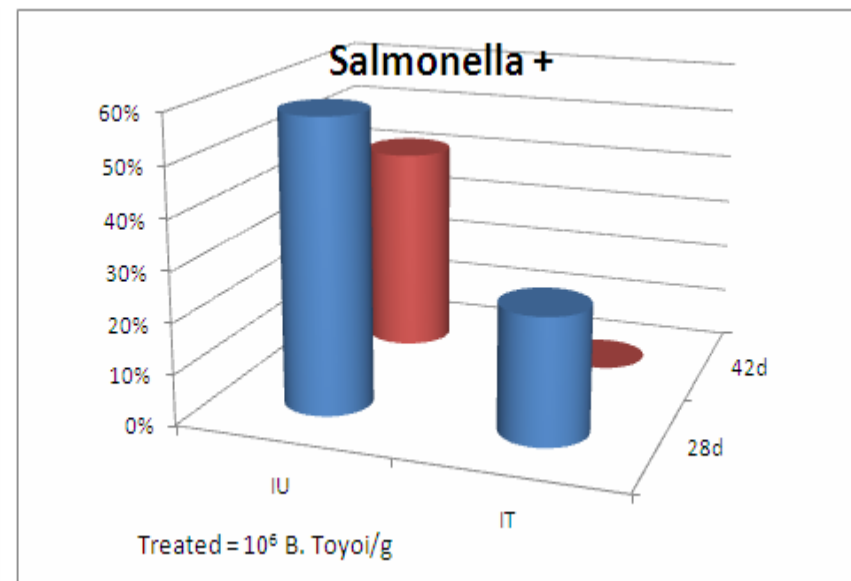
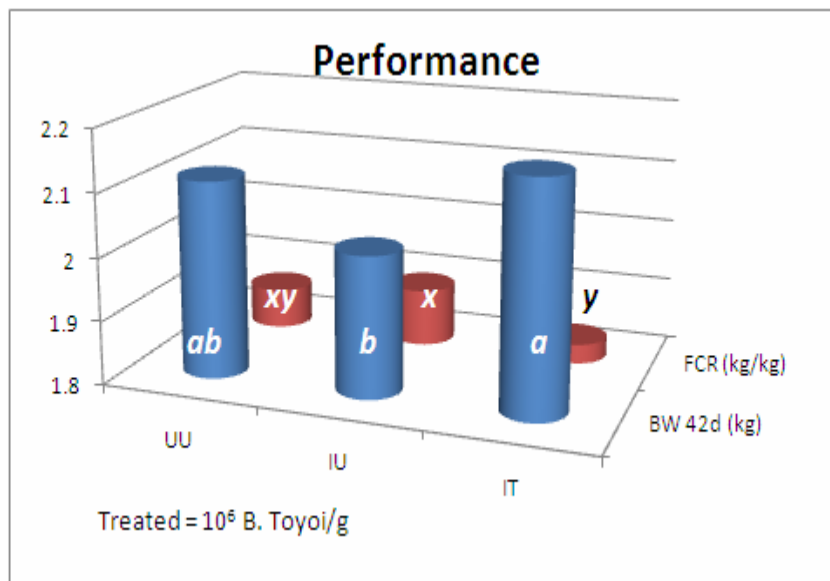
Effects of *Bacillus Subtilis* on live performance and carcass microbiological characteristics

	control	Calsporin	Probability	cv
BW 21d	665 b	690 a	++	8.60
BW 42 d	1967 b	2062 a	+++	7.93
FCR 42 d	1.780	1.759	NS	3.54
Mort %	1.58	2.08	NS	3.02
Salmonella	48/48	24/48	+++	7.82

Mean of 12 pens of 50 males and 12 pens of 50 females for control and 24 pens of 50 males and 24 pens of 50 females for Calsporin treatment.

Fritts et al 2000 Journal Applied Poultry Research

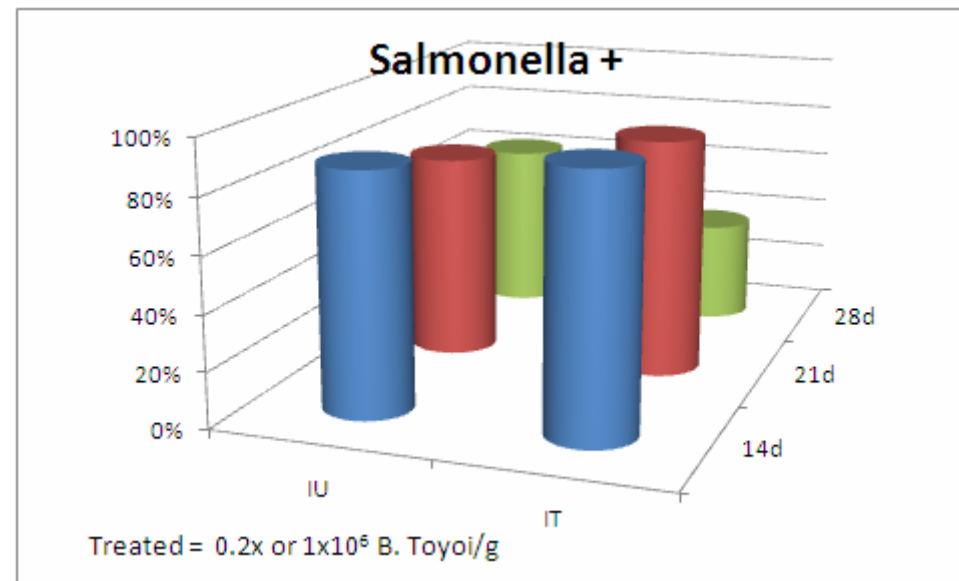
Effect of Toyocerin in broilers challenged with *Salmonella Enteritidis*.



1 Inoculated birds were given by gavage of 1 mL of PBS suspension containing 2×10^6 CFU *Salmonella enterica* var. *enteritidis* per mL (phage type 4, nalidixic acid resistant strain, field isolate, CReSA S3146) at day 3, 7 or 14.

Vila et al 2009 , Poultry Science

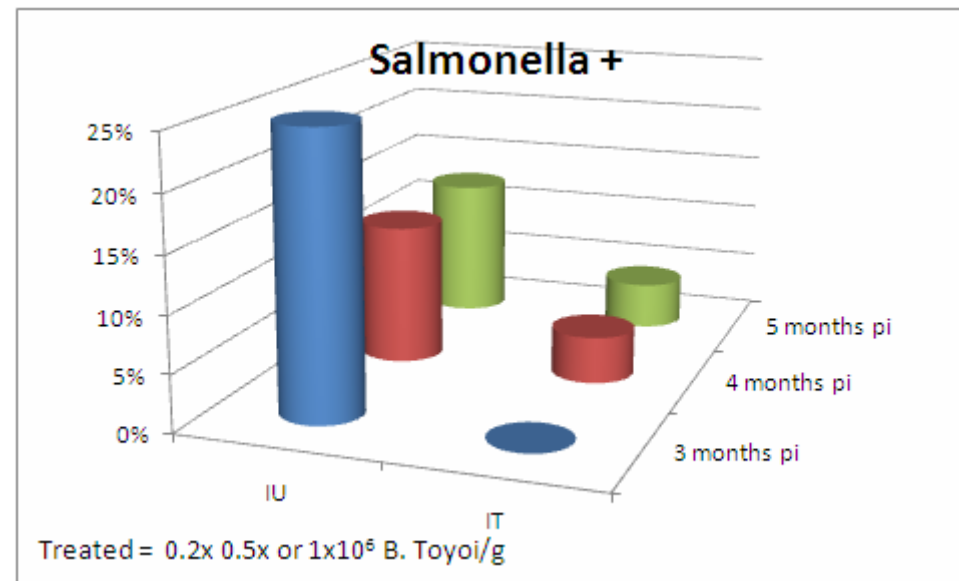
Effect of Toyocerin in Single Comb White leghorn chickens challenged with *Salmonella Enteritidis*.



1 Inoculated birds were given by gavage of 1 mL of PBS suspension containing 1x10⁸ CFU *Salmonella enterica* var. enteritidis per mL (phage type 4, nalidixic acid resistant strain, field isolate, CReSA GN825). at 7 days of age.

Vila et al 2009 , Poultry Science

Effects of Toyocerin in laying hens challenged with *Salmonella Enteritidis*



1 Inoculated birds were given by gavage of 1 mL of PBS suspension containing 1x10⁹ CFU *Salmonella enterica* var. *enteritidis* per bird (phage type 4, nalidixic acid resistant strains, field isolates, mixture of CReSA GN825 and CReSA GN1063) at 27 days on trial.

Some examples of efficacy .

Prebiotics



Effect of dietary prebiotic supplementation on the performance and immune response of broilers.

	Control	Avilamy cin	FOS 0.25	FOS 0.50	MOS 0.025	MOS 0.05	SEM
BW 4w	1348 c	1382 a	1384 a	1343 c	1361 b	1379a	3.66
FI 4w	2192	2229	2230	2191	2212	2225	20.5
FCR 4w	1.62	1.61	1.61	1.62	1.62	1.61	0.012
HL	0.92 a	0.83 ab	0.87 ab	0.92 a	0.82 ab	0.81b	0.031
IgG mg/mL	6.63	6.73	6.83	6.92	6.74	6.95	0.339
IgA mg/mL	5.86	5.98	5.86	5.75	5.91	5.99	0.451

Kim et al 2011 , Poultry
Science

Effects of FOS on performance, viable counts and morphology of intestinal mucosa (0-42 d)

FOS (g/kg)	0	2	4	8
BWG (g)	47.2 b	50.2 ab	52.5 a	49.4 ab
F/G	2.22 a	2.10 b	2.02 b	2.12 ab
Protease (U)	66 b	76 ab	84 a	77 ab
Lipase (U)	8.4 c	12.8 ab	14.8 a	10.7 bc
<i>Bifidobacterium</i>	7.2 b	7.8 ab	8.1 a	7.6 ab
<i>Lactobacillus</i>	7.5 b	8.1 ab	8.5 a	8.2 ab
<i>E. Coli</i>	7.0 b	6.5 ab	6.2 a	6.7 ab
Villus height (μm)	541 b	598 b	625 a	570 ab



Effects of dietary YCW and avilamycin wheat diets in broilers

	42 d				24 d	
	BW	DFI	FCR	Mort %	<i>E.coli</i>	<i>Lactobacillus</i>
Control	1877b	80.5b	1.844	3.3	6.41	8.03
Avilamycin	1959a	83.8a	1.839	1.7	5.90	8.28
YCW- Bak	1964a	83.9a	1.838	3.3	5.85	7.97
YCW -Bre	1887b	79.8b	1.819	4.1	5.68	7.73
SEM	20.3	1.04	0.01	1.7	0.33	0.31
Probability	0.05	0.05	0.72	0.77	0.46	0.68

Effects of dietary YCW and avilamycin Maize diets in broilers

	39 d				24 d	
	BW	DFI	FCR	Mort %	<i>E.coli</i>	<i>Lactobacillus</i>
Control	1950	82.0	1.678	6.1	6.44	7.54
Avilamycin	1999	81.9	1.633	3.0	6.37	7.80
YCW- Bak	2013	82.5	1.635	8.3	5.01	7.87
YCW -Bre	2012	83.9	1.666	8.3	6.15	7.39
SEM	26.9	1.73	0.02	1.50	0.54	0.40
Probability	0.34	0.82	0.46	0.10	0.25	0.81

40

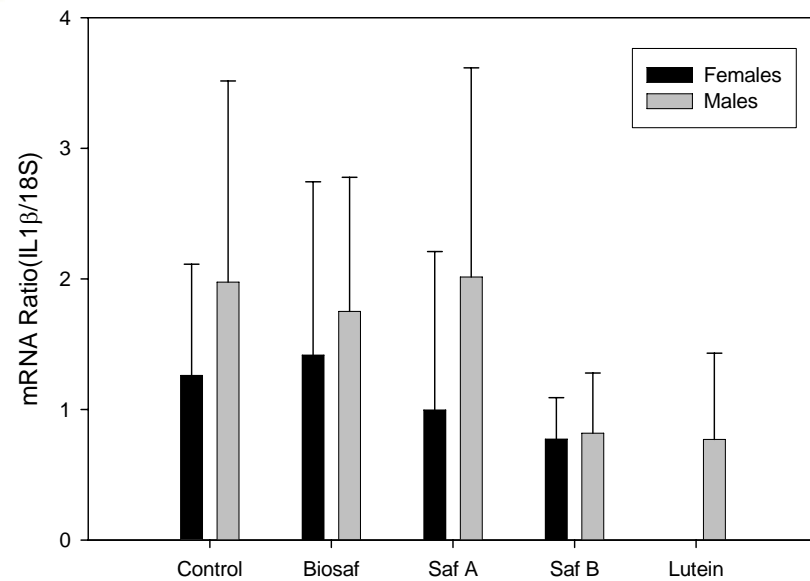
Use of yeast cell walls, B-1, 3/1, β -glucans and mannoproteins in broiler

	42 d				
	BW	DWG	DFI	FCR	Mort %
Control	2244	52.3	87.7	1.675	1.4
Avilamycin	2361	55.1	90.1	1.633	3.7
YCW	2313	54.0	89.4	1.654	5.8
MP	2284	53.3	87.7	1.645	4.3
BG	2312	53.9	89.0	1.650	1.4
MP+BG	2310	53.9	89.1	1.652	6.5
SEM	37.2	0.88	1.82	0.01	1.38
Probability	0.43	0.43	0.83	0.70	0.38

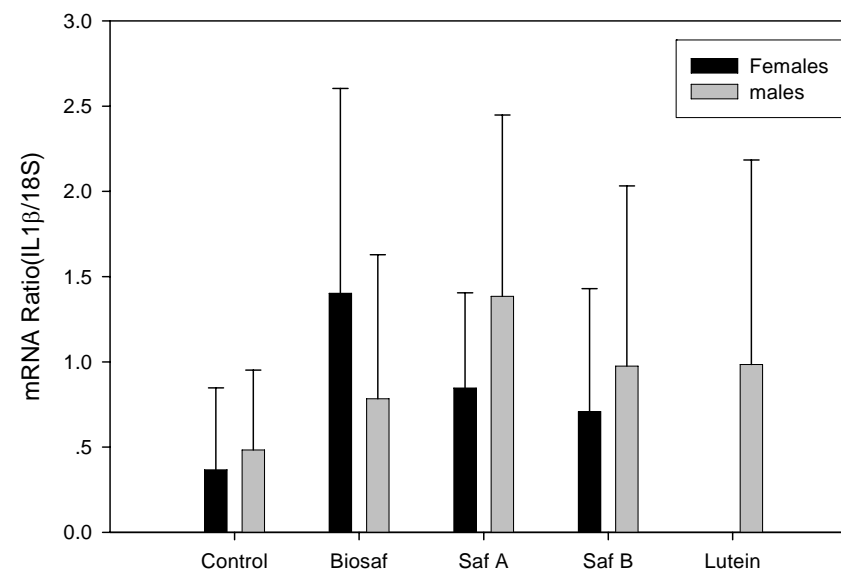
Use of yeast cell walls, B-1, 3/1, β -glucans and mannoproteins in broiler (experiment 2)

	42 d					
	BW	DWG	DFI	FCR	Mort %	Villus height(nm)
Control	2.404	56.8	93.1	1.660	13.2	957b
YCW	2.431	56.8	91.8	1.615	9.3	1159a
MP	2.419	56.5	90.4	1.600	12.7	1156a
BG	2.430	56.2	89.8	1.584	13.2	1090a
SEM	40.5	0.86	1.4	0.02	1.96	39.1
Probability	0.90	0.90	0.26	0.20	0.18	0.01

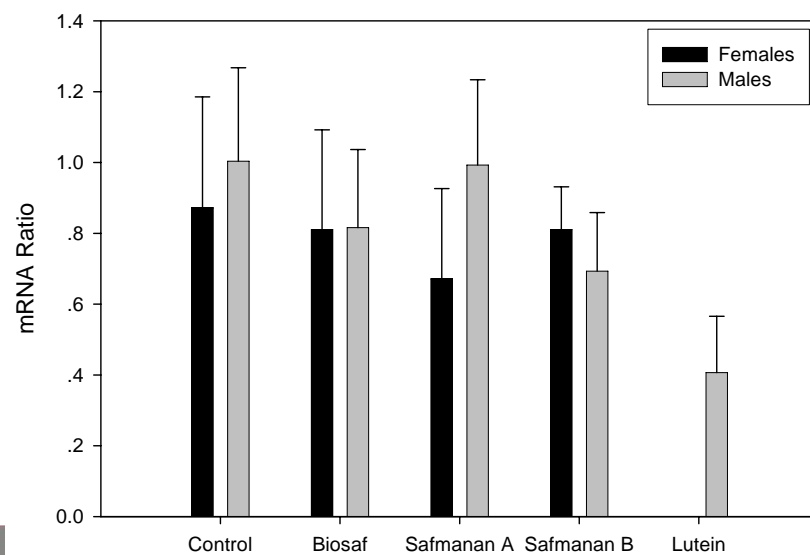
IL1 β Expression Ileum



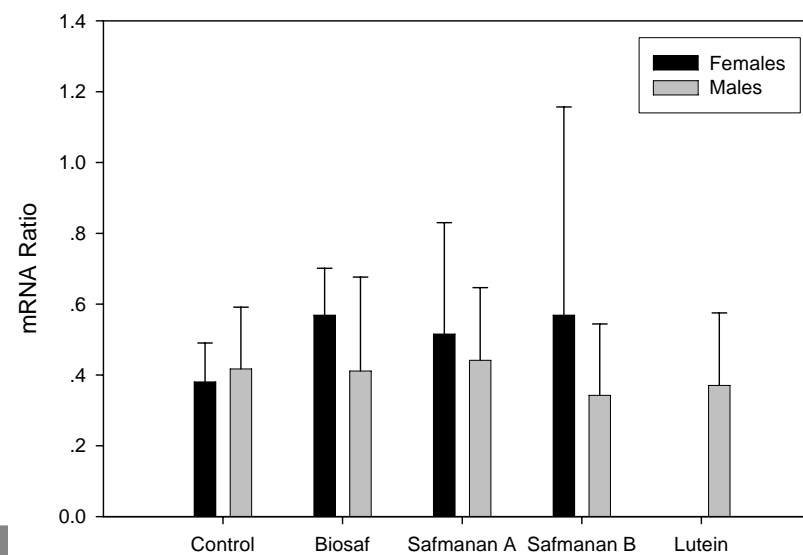
IL1 β Expression Colon



TLR2 Ileum



TLR2 Colon



IRTA-Patent on Prebiotics , Effects on Salmonella clearance. Carob gum

Ceratonia Silicua

PCT/EP2009/054172

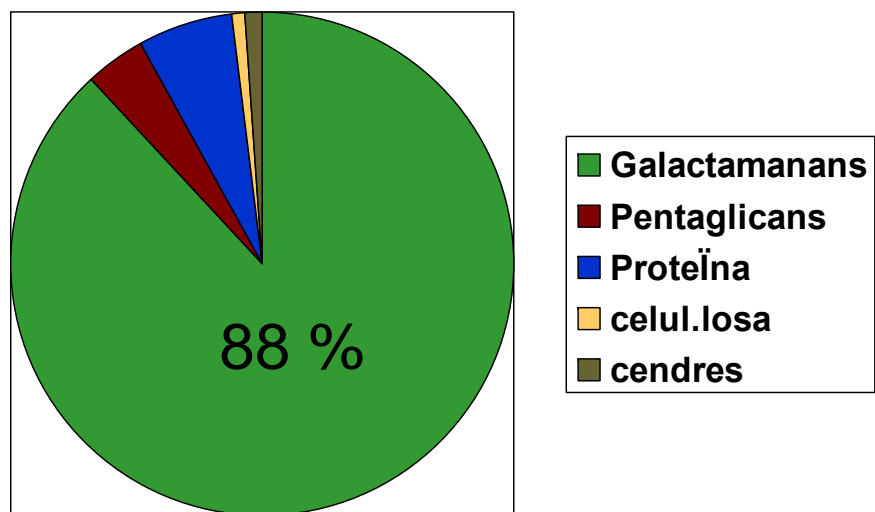
Salmosan®





WPSA-Italy , Forlì 7 04 2011

Carob gum composition



% on dry matter basis



mannose-galactose
4/1,

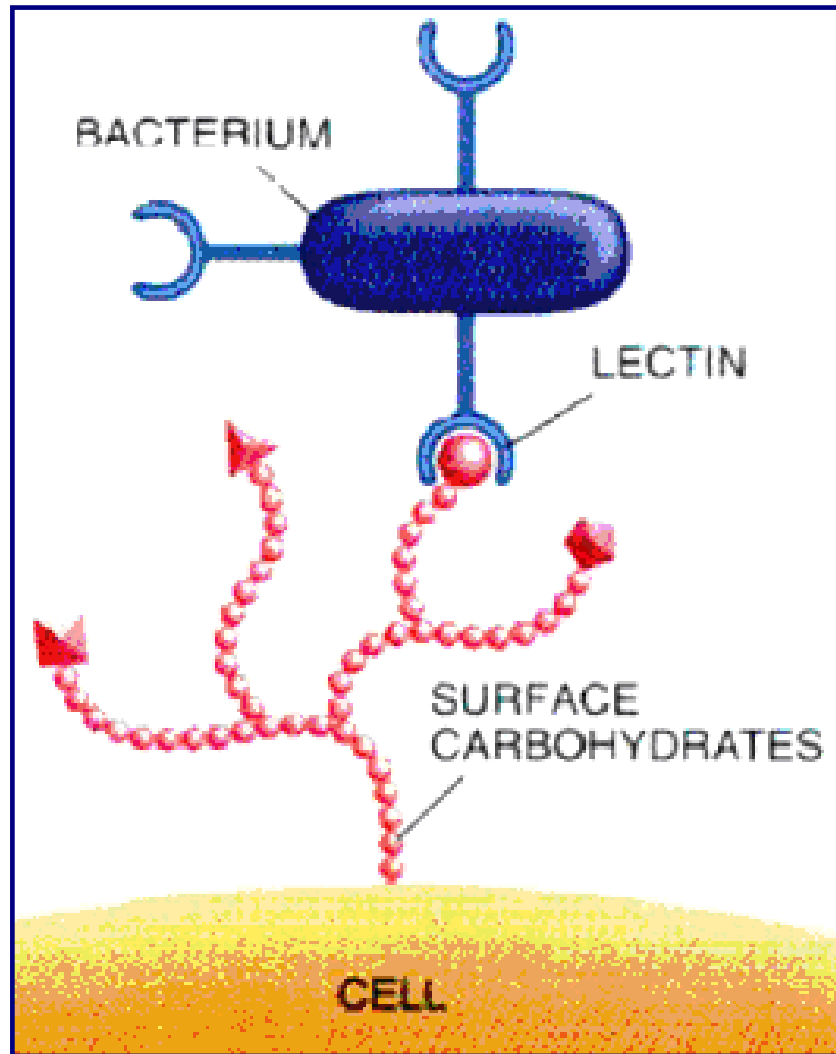
Substancias (substratos) con capacidad de prebiosis intestinal “Prebióticos”

Funcionalidad del goma de garrofin

**Reducir la adherencia de la Salmonella en el epitelio del tubo digestivo (Oyoyo et al. 1989),
interfiriendo en la adherencia de la fimbria tipo 1 en células intestinales (Duguid et al 1966)**

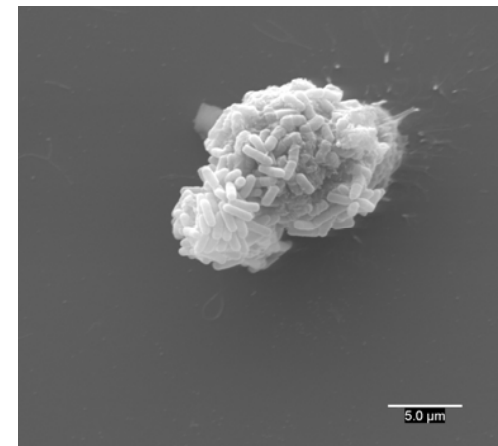
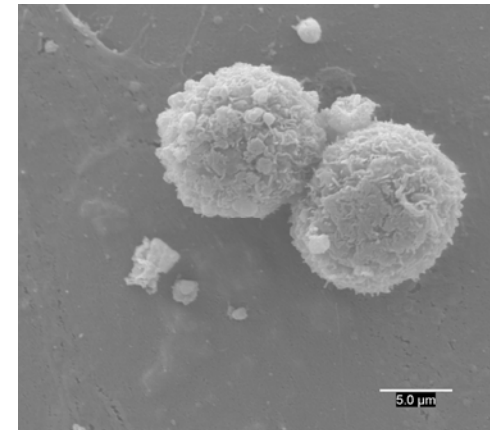
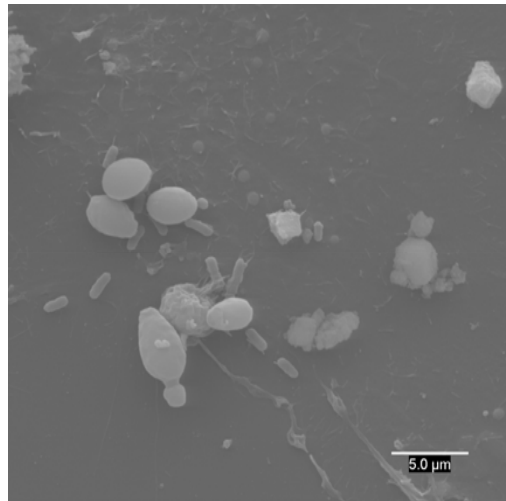
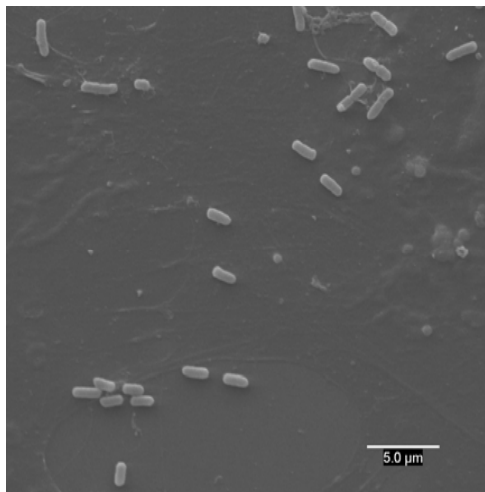
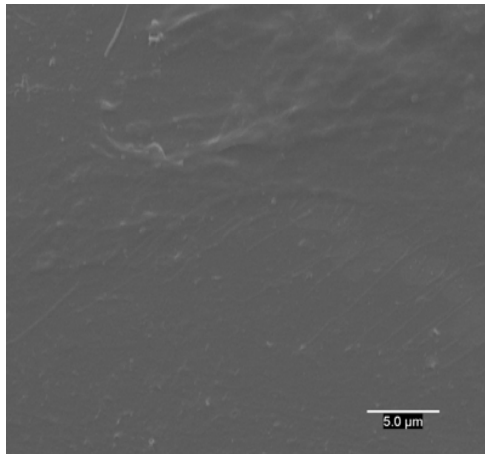
La presencia de manosa puede influir sobre la inmunidad innata con una mayor capacidad de pro-alarma ?

Exclusión competitiva

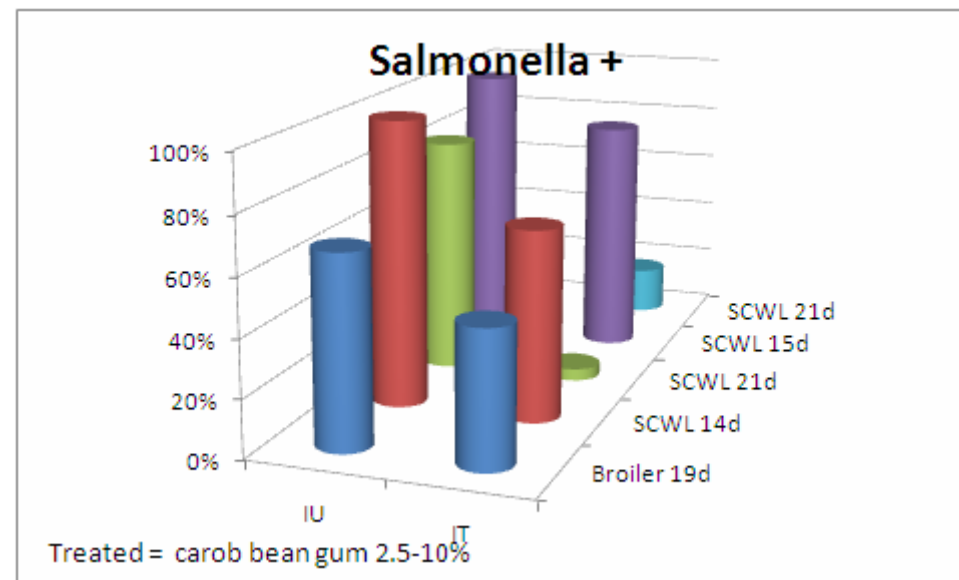


Los microorganismos potencialmente patógenos (p.e. *Salmonella*, *Escherichia coli* o *Vibrio cholerae*) emplean frecuentemente un grupo de proteínas y glicoproteínas de superficie denominadas "lectinas" con afinidad por la manosa (fimbrias tipo-I) a fin de unirse a ciertos carbohidratos de superficie localizados en las células del epitelio intestinal y colonizar así el entorno en que se encuentran tras fijarse a él

Salmonella aggregation by yeast and carob gum .



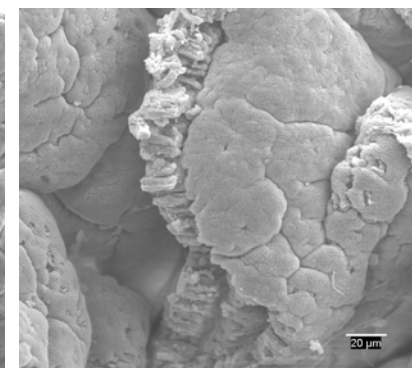
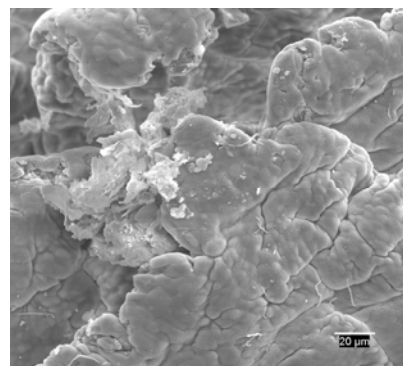
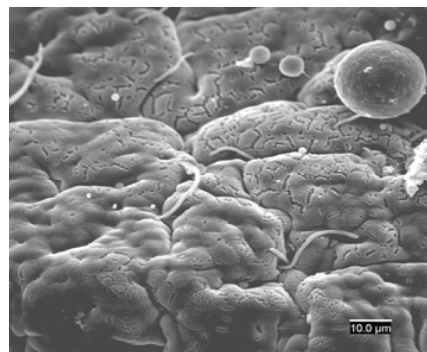
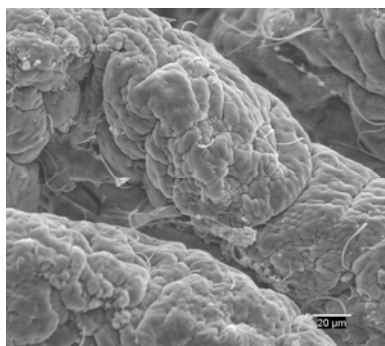
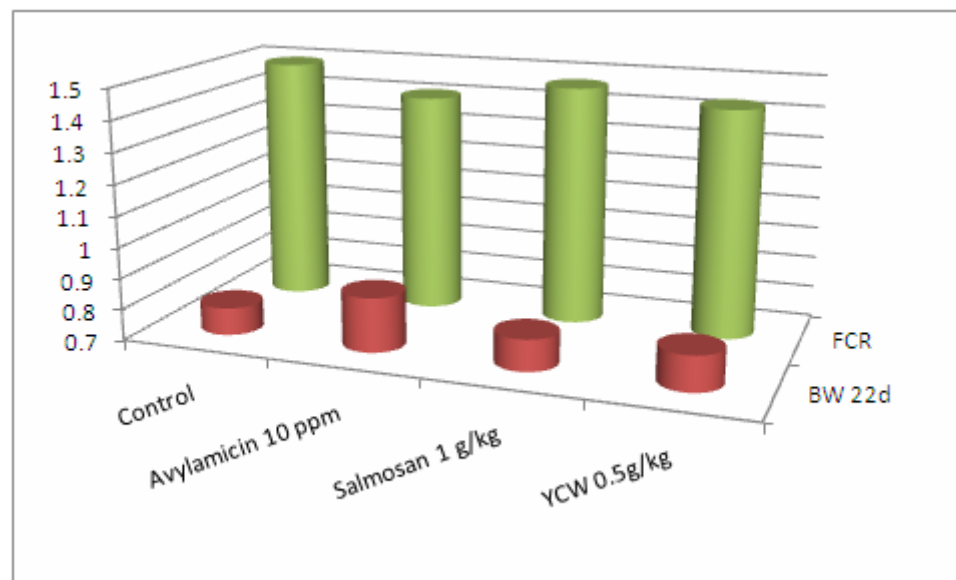
Effects of Carob bean gum on chickens challenged with *Salmonella Enteritidis*



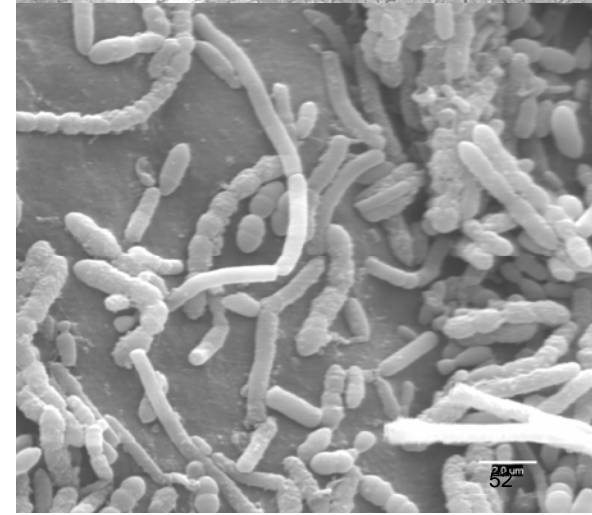
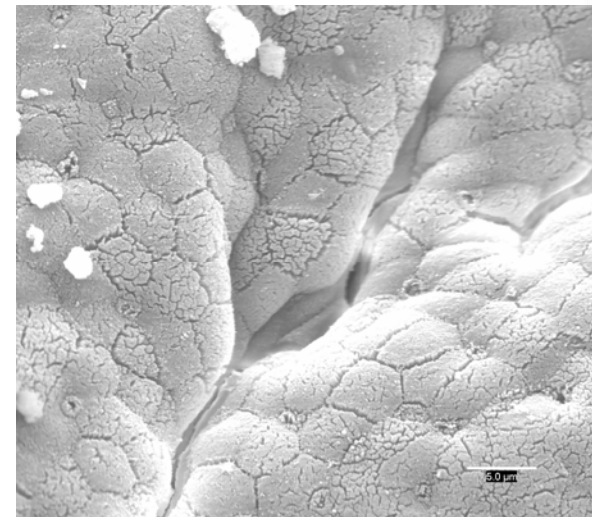
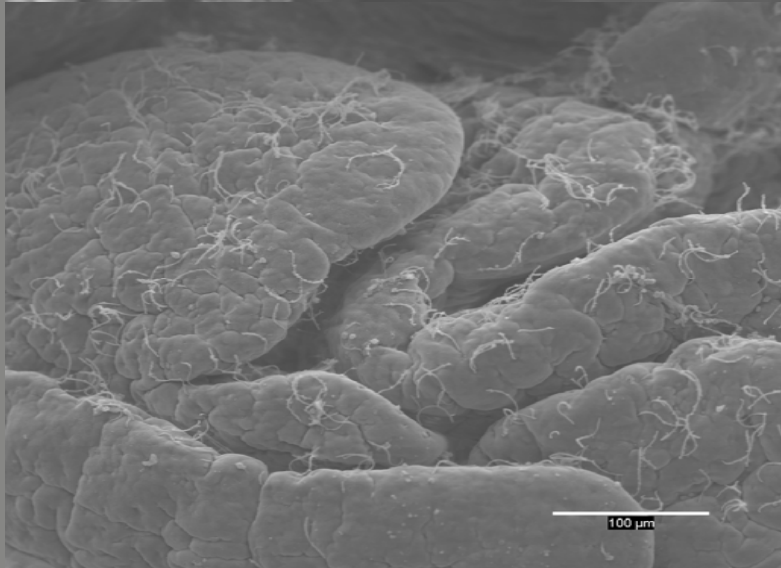
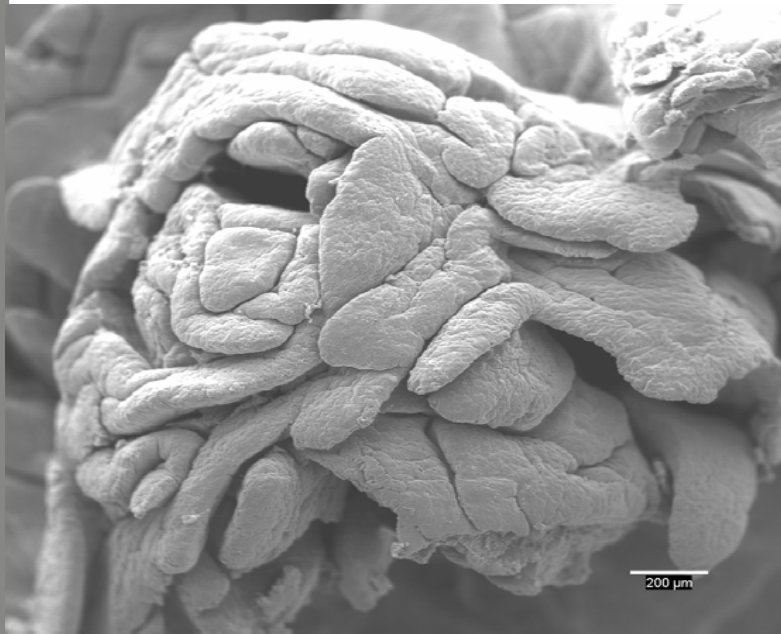
Inoculated birds were given by gavage 1 mL of PBS suspension containing 10⁶ CFU *Salmonella enterica* var. *Enteritidis* (phage type 4, nalidixic acid resistant strain, field isolate, CReSA S3146) at d 1

Vila et al 2011 (accepted Food Research International)

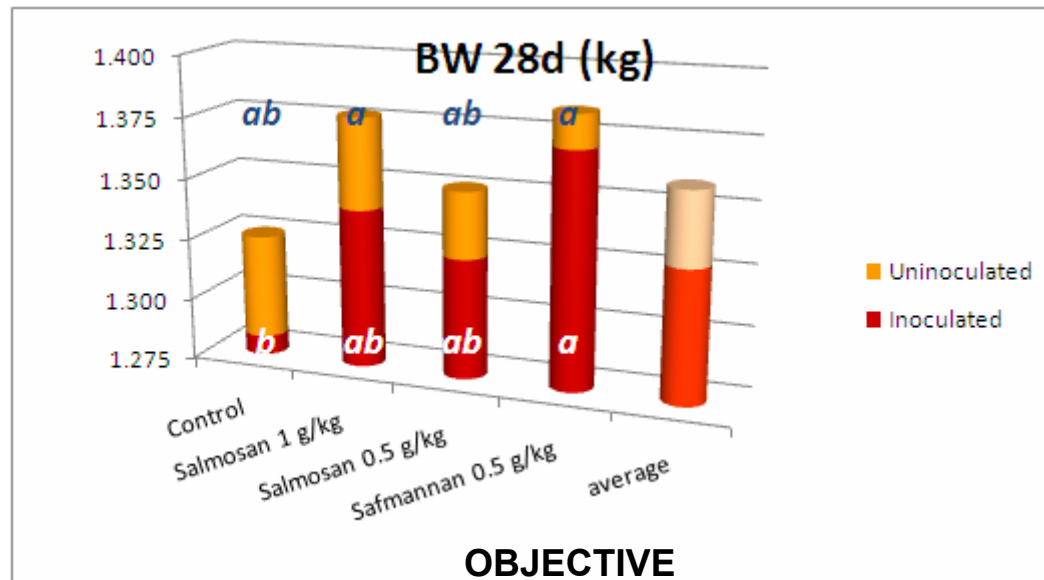
Effect of AGP vs galactomannans on broiler performance and gut morphology



Cecal Tonsil of Chickens of 21 days

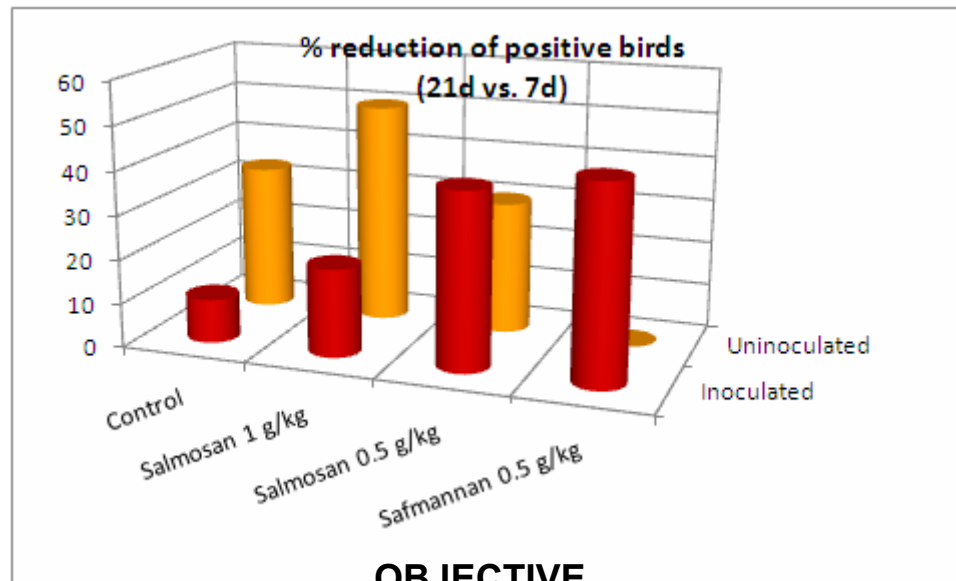


Controlling *Salmonella* action in poultry using bacterial blocking natural substances in feed



To evaluate the effect of dietary carob bean (Salmosan®) and yeast cell wall (Safmannan®) on broiler chicken performance and *Salmonella* incidence when natural mechanisms of infection were simulated

Controlling *Salmonella* action in poultry using bacterial blocking natural substances in feed



To evaluate the effect of dietary carob bean (Salmosan®) and yeast cell wall (Safmannan®) on broiler chicken performance and *Salmonella* incidence when natural mechanisms of infection were simulated

Many Thanks for your attention

Pilot feed manufacturing at IRTA

